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Contrasting mechanisms underlie short- and longer-term soil respiration responses to experimental warming in a dryland ecosystem

Running head: Warming effects on soil respiration

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Abstract

Soil carbon losses to the atmosphere through soil respiration are expected to rise with ongoing temperature increases, but available evidence from mesic biomes suggests that such response disappears after a few years of experimental warming. However, there is lack of empirical basis for these temporal dynamics in soil respiration responses, and for the mechanisms underlying them, in drylands, which collectively form the largest biome on Earth and store 32% of the global soil organic carbon pool. We coupled data from a ten-year warming experiment in a biocrust-dominated dryland ecosystem with laboratory incubations to confront 0-2 years (short-term hereafter) vs. 8-10 years (longer-term hereafter) soil respiration responses to warming. Our results showed that increased soil respiration rates with short-term warming observed in areas with high biocrust cover returned to control levels in the longer-term. Warming-induced increases in soil temperature were the main driver of the short-term soil respiration responses, whereas longer-term soil respiration responses to warming were primarily driven by thermal acclimation and warming-induced reductions in biocrust cover. Our results highlight the importance of evaluating short and longer-term soil respiration responses to warming as a mean to reduce the uncertainty in predicting the soil carbon–climate feedback in drylands.

Keywords: soil respiration, biocrusts, dryland, microbial thermal acclimation, short-term vs longer-term warming, soil temperature, soil moisture

Introduction

Soil respiration is expected to increase with global warming (Davidson & Janssens, 2006; Kirschbaum, 2006), contributing to enhance atmospheric CO₂ concentration. Thus, warming-induced soil carbon (C) losses via soil respiration may lead to a positive C cycle–climate feedback (Tucker, Bell, Pendall, & Ogle, 2013), which is indeed embedded into the climatic models of the IPCC (Ciais et al., 2014). However, most experiments conducted to date on this topic have typically lasted less than four years (Wang et al., 2014), and there is growing evidence showing that elevated soil respiration rates may gradually be offset towards ambient values after a few years of experimental warming (Kirschbaum, 2004; Luo, Wan, Hui, & Wallace, 2001; Melillo et al., 2017, 2002). Multiple mechanisms have been hypothesized to explain such transient effects of warming on soil respiration. For instance, the thermal acclimation of soil microorganisms to the ambient temperature regime (Bradford et al., 2019; Dacal, Bradford, Plaza, Maestre, & García-Palacios, 2019) and the depletion of labile soil C sources (Hartley, Hopkins, Garnett, Sommerkorn, & Wookey, 2008; Schindlbacher, Schnecker, Takriti, Borken, & Wanek, 2015) may drive soil respiration responses to warming over time. Additionally, given that soil temperature and moisture are the most important controls on soil respiration (Conant, Dalla-Betta, Klopatek, & Klopatek, 2004), warming-induced changes in microclimatic variables may alter soil microbial activity, leading to shifts in soil respiration rates (Luo et al., 2001). The lack of consensus on the relative importance of these mechanisms hinders our ability to model longer-term soil respiration responses to warming, which are fundamental to increase confidence in soil C projections in a warmer world (Bradford et al., 2016; Zhou et al., 2012).

Beyond heterotrophic microbial CO₂ production, soil respiration is also a product of plant roots and other autotrophic organisms inhabiting soil surfaces such as biological soil crusts (biocrusts hereafter; topsoil communities formed by cyanobacteria, algae, mosses, liverworts, fungi, bacteria and lichens; Weber, Büdel, & Belnap, 2016). In drylands, which cover 41% of the total land surface (Cherlet et al., 2018) and store 32% of the Earth's soil organic C (SOC) pool (Plaza et al., 2018), up to 42% of the overall soil respiration comes from biocrust-dominated microsites (Castillo-Monroy, Maestre, Rey, Soliveres, & García-Palacios, 2011; Feng et al., 2014, 2013). Biocrusts are particularly relevant for the global C cycle, as it has been estimated that they cover ca. 12% of the Earth's terrestrial surface (Rodríguez-Caballero et al., 2018) and fix over 2.6 Pg·yr⁻¹ of atmospheric C globally (Elbert et al., 2012). Given their extent and importance for the C

cycle, biocrusts are a major ecosystem component when evaluating warming effects on soil respiration in drylands.

Biocrust constituents are severely affected by warming; the physiological performance of biocrust-forming lichens and mosses have been found to decrease with warming in experiments conducted in Spain, USA, China and South Africa (Grote, Belnap, Housman, & Sparks, 2010; Maestre, Delgado-Baquerizo, Jeffries, Eldridge, & Ochoa, 2015; Maestre et al., 2013; Maphangwa, Musil, Raitt, & Zedda, 2012; Ouyang & Hu, 2017). These responses have been linked to warming-induced reductions in soil moisture and dew events (Ladrón de Guevara et al., 2014; Ouyang & Hu, 2017). Such water sources are critical to maintain the photosynthetic activity of biocrust communities (del Prado & Sancho, 2007; Veste, Littmann, Friedrich, & Breckle, 2001), and warming-induced alterations on them may lead to dramatic losses in the cover of biocrust-forming lichens and mosses (up to 40%) after a few years of temperature manipulation (Darrouzet-Nardi, Reed, Grote, & Belnap, 2018; Escolar, Martínez, Bowker, & Maestre, 2012; Ferrenberg, Reed, Belnap, & Schlesinger, 2015; Maestre et al., 2013). Given the importance of biocrust cover for soil respiration in drylands (Castillo-Monroy, Maestre, et al., 2011; Feng et al., 2014, 2013), the heterotrophic mechanisms (i.e. substrate depletion, microbial acclimation and changes in microclimatic variables) driving soil respiration responses to warming should be assessed jointly with the shifts in biocrust cover promoted by elevated temperatures (García-Palacios et al., 2018; Maestre et al., 2013).

In drylands, most studies evaluating soil respiration responses to experimental warming have been conducted over short-term periods (Darrouzet-Nardi, Reed, Grote, & Belnap, 2015; Escolar, Maestre, & Rey, 2015; Guan, Li, Zhang, & Li, 2019; Maestre et al., 2013) and consequently, longer-term warming effects are virtually unknown. To our knowledge, only Darrouzet-Nardi et al., (2018) have explicitly confronted short- vs. longer-term soil respiration responses to warming in biocrust-dominated drylands, but no study so far has addressed the heterotrophic and autotrophic mechanisms underlying transient soil respiration responses to warming. Given the importance of soil carbon-climate feedbacks to forecast greenhouse gas emissions globally (Carey et al., 2016; Crowther et al., 2016), and the extent of drylands worldwide, it is critical to evaluate both short and longer-term soil respiration responses to warming in these environments and how these are modulated by biocrusts and soil microbial communities.

Here, we confronted 0-2 years (short-term, hereafter) vs. 8-10 years (longer-term, hereafter) soil respiration responses to experimental warming in a biocrust-dominated dryland in central Spain. Data from this experiment were coupled to those from laboratory incubations at four assay temperatures (10, 20, 30 and 40°C), which allowed us to gain mechanistic insights on the importance of autotrophic and heterotrophic pathways as drivers of soil respiration responses to warming over time. Using this combination of approaches, which to the best of our knowledge has not been used before when evaluating soil respiration responses to warming in drylands, we evaluated: i) short- and longer-term warming impacts on soil respiration and its temperature sensitivity (objective i), ii) how warming-induced effects on soil temperature and moisture affect soil respiration responses to elevated temperatures (objective ii), iii) the role of biocrusts as modulators of short- and longer-term soil respiration responses to warming (objective iii), and iv) the importance of thermal acclimation of soil microbial respiration as a driver of soil respiration responses to longer-term warming (objective iv).

Materials and methods

Study area

The study was conducted at the Aranjuez Experimental Station, located in central Spain (40°02'N–3°32'W; elevation = 590 m a. s. l.). Its climate is Mediterranean semiarid, with mean annual temperature and precipitation values (2008-2018 period) of 16,5°C and 336 mm, respectively. The soil is a Gypsic Leptosol (IUSS Working Group WRB, 2006). Perennial plant cover is < 40%, and biocrust communities dominated by lichens such as *Diploschistes diacapsis*, *Squamaria lentigera*, *Fulgensia subbracteata* and *Buellia zoharyi* and mosses *Pleurochaete squarrosa* and *Didymodon acutus* cover ~32% of the soil surface (Castillo-Monroy, Maestre, et al., 2011; Maestre et al., 2013). Cyanobacteria from the genera *Microcoleus*, *Schizothrix*, *Tolypothrix*, *Scytonema* and *Nostoc* also form part of biocrusts at this site (Cano-Díaz, Mateo, Muñoz-Martín, & Maestre, 2018).

In July 2008, we established a full factorial experiment with two treatments of two levels each: warming (ambient vs. increased temperature) and initial biocrust cover (low: < 20% vs high: >50%). We installed open top chambers (OTCs) to reach a warming scenario similar to the temperature increase of 2–3°C forecasted for 2040–2070 in this region in atmosphere-ocean general circulation models (De Castro, Martín-Vide, & Alonso, 2005). OTCs present a hexagonal design made of methacrylate sheets (40 × 50 × 32 cm), a material that according to the

manufacturer (Decorplax S.L., Humanes, Spain) ensures 92% transmittance in the visible spectrum and very low emission in the infrared wavelength. To allow air circulation and thus to avoid overheating, OTCs are suspended 3–5 cm over the ground by a metal frame. Ten plots (1.25 x 1.25 m) per combination of treatments were randomly distributed and separated at least 1 m to diminish the risk of lack of independence between replicates ($n = 40$). We inserted a PVC collar (20 cm diameter, 8 cm height) in each plot to monitor soil respiration and biocrust cover over time. See Escobar et al. (2012) and Maestre et al. (2013) for additional details on the experimental design.

Testing the warming effects on soil microclimatic conditions

We focused on warming effects on soil temperature and soil moisture as the two main drivers controlling soil respiration in drylands (Castillo-Monroy, Maestre, et al., 2011; Conant et al., 2004; Veste et al., 2001). In parallel to soil respiration measurements, we monitored soil temperature at 0-2 cm depth with protected diodes at the beginning of the experiment and since 2012 (i.e. four years after experimental set-up) with a Li-8100 Automated Soil CO₂ Flux System (Li-COR, Lincoln, NB, USA) because the later measurements were faster and more accurate. Data obtained with the Li-8100 were corrected using a calibration between both methods ($r=0.956$, Figure S1). Volumetric soil moisture was measured monthly at 0-5 cm depth using time-domain reflectometry (TDR; Topp & Davis, 1985) in every plot at the same time of soil respiration measurements. Additionally, and given that at our study site soil moisture dynamics is largely driven by specific pulses (Berdugo, Soliveres, & Maestre, 2014; Lafuente, Berdugo, Ladrón de Guevara, Gozalo, & Maestre, 2018) soil moisture (0-5 cm depth) was monitored continuously every 2.5 hours using automated sensors (ECH₂O humidity sensors, Decagon Devices Inc., Pullman, USA). Specifically, we used four replicates for control plots (all corresponding to high initial biocrust cover) and six for warming plots (half of them for each initial biocrust cover level). Sensors to monitor soil moisture continuously were installed in February 2009.

Soil CO₂ efflux measurements and its temperature sensitivity (Q_{10})

The soil CO₂ efflux rate of the whole soil column, including both biocrusts and soil microbial communities, was measured *in situ* once a month in two contrasting periods: short-term (0-2 years) and longer-term (8-10 years) after the setup of the experiment. Measurements were conducted with a closed dynamic system (Li-8100). The opaque chamber used for these

measurements had a volume of 4843 cm³ and covered an area of 317.8 cm². Given the low CO₂ efflux rates typically observed in semiarid ecosystems (Castillo-Monroy, Maestre, et al., 2011; Maestre et al., 2013; Rey et al., 2011), sampling period was set-up to 120 s to ensure reliable measurements. We included 45 seconds of purge after each soil CO₂ efflux measurement.

Furthermore, we established a deadband of 15 seconds once the chamber was closed when no flux was recorded to allow biocrusts to acclimate to dark. In every survey, half of the replicates were measured in one day (between 10:00 and 13:00 local time), and the other half were measured over the next day in the same period. Annual plants were removed from the PVC collars at least 48 hours before soil respiration measurements.

We evaluated the temperature sensitivity of soil respiration using Q₁₀, defined as the increment in soil respiration when temperature increases by 10°C and calculated at each plot using the following equations (Luo & Zhou, 2006):

$$R_s = R_0 e^{\beta t} \quad (1)$$

Where R_s is soil respiration (μmol m⁻² s⁻¹), R₀ is the basal soil respiration rate (μmol m⁻² s⁻¹) or intercept of soil respiration at 0°C, and t is soil temperature (in °C) measured at the same time as R_s. β was used to calculate the Q₁₀ (increment in R_s when t increases by 10 °C) as follows:

$$Q_{10} = e^{10\beta} \quad (2)$$

Monitoring changes in biocrust cover with warming

The total cover of the two major visible components of the biocrust community (lichens and bryophytes) was estimated in each PVC collar at the beginning of the experiment and on a yearly basis thereafter (except during the second year of the experiment, when these measurements were not taken). We used high-resolution photographs to assess the proportion of each collar covered by these biocrust components using the software GIMP (<http://www.gimp.org/>, to map biocrust-covered areas) and ImageJ (<http://rsb.info.nih.gov/ij/>, to calculate the size of biocrust-covered areas). Cover estimates obtained with this method are highly related (r²=0.84) to those observed in the field with the point sampling method (Ladrón de Guevara et al., 2018). For this study we only considered biocrust surveys included within our sampling periods. Therefore, we used the surveys conducted 1 yr and 9-10 yr (average of both surveys) after the setup of the experiment for the short- and longer-term periods, respectively.

Addressing thermal acclimation of soil microbial respiration using laboratory incubations

We sampled soils (0-5 cm depth) in five field replicates per combination of treatments in 2017, nine years after the setup of the experiment. Biocrust visible components were removed from the samples, which were sieved at 2 mm mesh and stored at 4 °C for a couple of days until laboratory incubation. We conducted short-term (10 h) laboratory soil incubations at four assay temperatures (10, 20, 30 and 40°C) as performed in similar mechanistic tests of thermal soil microbial acclimation (Atkin & Tjoelker, 2003; Bradford, Watts, & Davies, 2010; Hochachka & Somero, 2002; Tucker et al., 2013). Soil incubations were performed at 60% of water holding capacity, dark conditions and 100% air humidity.

For each soil sample, we measured soil respiration rates after the addition of two different substrates: sterile deionized water and glucose (at a dose of 10 mg C g⁻¹ dry soil) using the MicroResp™ technique (Campbell, Chapman, Cameron, Davidson, & Potts, 2003). The former substrate was used to determine soil basal respiration, whereas the latter was used to account for the effect of substrate limitation on soil respiration rates (Bradford et al., 2010). The glucose dose used in this study is considered to exceed microbial demand (Davidson, Janssens, & Luo, 2006). The MicroResp™ technique (Campbell et al., 2003) is a high-throughput colorimetric method measuring soil respiration rates. We used a CO₂ detection solution containing cresol red indicator dye that experiences a colour change because of the variation in pH occurring when respired CO₂ reacts with the bicarbonate of the detection solution. Each microplate well was filled with 150 µl aliquots of the detection solution and was attached to the deep-well microplates containing the soil samples (0.5 g fresh soil/well). Both plates were incubated together at the assay temperature (10, 20, 30 or 40°C) during the last five hours of the incubation period to avoid detection solution saturation. The detection plate colour development was read immediately before and after the last five hours of the incubation at 595nm. The colour change in the detection solution was calibrated with an alkali trapping method ($r^2=0.86$, $P<0.001$, Lundegardh, 1927, Figure S2). The absorbance values observed in our study fell into the most flattened part of the calibration graph (i.e. between 0.3 and 0.6), suggesting that the detection solution was not saturated.

It is necessary to control for microbial biomass to test for thermal acclimation (Bradford et al., 2010). All available methods to estimate soil microbial biomass have drawbacks (Bradford et al., 2008, 2009; Hartley, Hopkins, Garnett, Sommerkorn, & Wookey, 2009), and hence we measured soil microbial biomass using three different methods to increase the robustness of our results. First, we measured soil induced respiration (µg CO₂-C g soil⁻¹ h⁻¹) using autolyzed yeast (Yeast-SIR) as a substrate at 20°C (Fierer, Schimel, & Holden, 2003). Yeast was added at a dose

of 1 mL g soil⁻¹ (dry weight equivalent) from a solution containing 12 g of yeast L⁻¹ of water. Second, we used a chloroform-fumigation extraction (CFE) (Vance, Brookes, & Jenkinson, 1987). Specifically, we prepared two replicates per sample with almost the same amount of soil: one of the replicates was fumigated with chloroform and the other one remained as a control. Then, we measured total organic carbon (TOC) with an automated TOC analyser in K₂SO₄-diluted soil samples. The microbial biomass estimation derived from this technique (mg C kg soil⁻¹) was calculated by the difference between fumigated and unfumigated samples. Finally, we measured the relative abundance of soil bacteria (number of DNA copies g⁻¹ soil) using qPCR. The bacterial 16S-rRNA genes were amplified with the Eub 338-Eub 518 primer sets as described in Maestre et al. (2015).

Statistical analyses

We conducted a series of statistical analyses to achieve the different objectives of the study. To achieve objective i (i.e. how short- and longer-term warming affect soil respiration and its temperature sensitivity), we built linear mixed-effect regression models (LMMs) that included warming, initial biocrust cover and their interaction as fixed factors. The temporal dependence of soil respiration measurements across replicates over time (i.e. repeated measures) was tested by including replicate identity and sampling date in the model as random factors. To test the effect of warming on Q₁₀, we built linear regression models (LMs) including warming, initial biocrust cover and their interaction as fixed factors.

Soil respiration missing data due to technical issues (11% of the data equally distributed between both sampling periods) was imputed using the R package missForest (Stekhoven & Bühlmann, 2012) as it was done in similar studies (Darrouzet-Nardi et al., 2015, 2018). To confirm that data imputation did not artificially alter the treatment effects, we repeated the same LMMs using the original data with missing values. We conducted the analyses testing the effect of warming on soil respiration and temperature sensitivity (Q₁₀) using both the original data with missing values and the filled data. The missForest is an iterative imputation algorithm based on random forest models, which are considered ensemble-learning methods (Breiman, 2001). The algorithm starts filling the missing data with the variable with the fewest gaps and then iteratively re-fits new imputation models until a stopping criterion is reached. We fit one separated missForest model for each combination of treatments (i.e. four models in total) including soil respiration, temperature and moisture, biocrust cover and sampling date.

To achieve objective ii (how warming-induced impacts on soil temperature and moisture affect soil respiration responses to this climate change driver), we first evaluated the effects of warming on soil temperature and moisture measured monthly using LMMs that included warming, initial biocrust cover and their interaction as fixed factors. The temporal dependence of soil temperature and soil moisture measured monthly across replicates over time (i.e. repeated measures) was tested by including replicate identity and sampling date in the model as random factors. Additionally, we performed a similar analysis for soil moisture measured continuously, but for both biocrust cover levels together, as we lack enough replicates to separate between low and high cover. To compare the effect of warming on soil moisture measured monthly and continuously, we only consider soil moisture monthly measurements conducted once both sampling methods were available (i.e. since February 2009). We used the monthly mean of continuous soil moisture data for doing so. We then calculated the effect size of warming on soil respiration, temperature and moisture measured at each plot on a monthly basis for each period (i.e. 0-2 yr and 8-10 yr) using the response ratio (RR, Hedges, Gurevitch, & Curtis, 1999):

$$RR (\text{soil respiration}) = \ln (RSW / RSC) \quad (3)$$

where RSW is the soil respiration in each warmed plot and RSC is the mean soil respiration in the control plots. The RRs were estimated separately for each initial biocrust cover level. To calculate the RRs, we first computed the average across the sampling dates per period for each plot. Then, to test how warming-induced changes in soil temperature impacted soil respiration, we evaluated the relationship between the RR of soil respiration and that of soil temperature using LMs. Similarly, to address the relationship between warming-induced changes in soil moisture and soil respiration, we evaluated the relationship between the RR of soil respiration and that of soil moisture using LMs.

To achieve objective iii (role of biocrusts as modulators of short- and longer-term soil respiration responses to warming), we first evaluated the effects of warming on the biocrust cover using LMs with warming, initial biocrust cover and their interaction as fixed factors. Then, we evaluated whether warming-induced changes in biocrust cover control soil respiration responses to warming during short- and longer-term periods. To do so, we evaluated the relationship between the RR of soil respiration and that of biocrust cover using LMs. The RRs were calculated as described above for soil respiration.

To achieve objective iv (importance of thermal acclimation by soil microbial respiration), we tested whether soil heterotrophic microbial respiration acclimates to elevated temperatures

after longer-term warming. To do so, we first evaluate the effect of warming on soil microbial biomass in the longer-term. For that purpose, we used LMs that include warming, initial biocrust cover and their interaction as fixed factors. We fit a separate model for each of the three methods used to estimate microbial biomass (i.e. Yeast-SIR, CFE and qPCR). Then, we statistically controlled for differences in microbial biomass by including it as a covariate in the model (substrate limitation was alleviated in the laboratory incubations using glucose in excess of microbial demand) as conducted in previous studies (Bradford et al., 2019, 2010; Dacal et al., 2019). We used this approach to control for microbial biomass instead of the mass-specific respiration (ratio between soil respiration and microbial biomass), as ratios may obscure true relationships among variables (Bradford et al., 2019; Jasienski & Bazzaz, 1999). Additionally, some previous thermal adaptation studies found the same results using either mass-specific respiration or SIR and microbial biomass as a covariate in the model (Bradford et al., 2008, 2010). This suggests that this covariate approach is also appropriate to control the effect of changes in microbial biomass when testing for thermal acclimation of soil respiration. Therefore, we followed the covariate approach as it was an appropriate method to achieve this objective; by doing so we also avoided the problems associated with including ratios in statistical models. Specifically, we ran a separate LM for each soil microbial biomass estimation method (i.e. Yeast-SIR, CFE and qPCR). These LMs incorporated warming, initial biocrust cover, the interaction of these two treatments, assay temperature and soil microbial biomass as fixed factors, and analysed their effects on potential soil microbial respiration. The interaction between assay temperature and the warming treatment was also tested but removed because it was not significant ($p=0.860$). To represent the results of these thermal adaptation analyses (i.e. Figure 5), we followed an approach that estimates soil respiration rates considering all the variables included in the models (i.e. warming treatment, initial biocrust cover, the interaction between these two treatments, assay temperature and microbial biomass. Specifically, we estimated the relative effect size of each of these variables on potential soil microbial respiration rates using the unstandardized regression parameters. These effect sizes depend on the slope coefficient for the specific variable obtained by fitting the LMM and on the observed variation in the values of that variable. To discern the effect of warming, we allowed their values to vary across both levels of this treatment (i.e. control and warming) while holding the remaining variables constant at the mean of all observations for each variable.

All the statistical analyses were conducted using the R 3.3.2 statistical software (R Core Team, 2015). The LMMs were fit with a Gaussian error distribution using the 'lmer' function of the lme4 package (Bates, Mächler, Bolker, & Walker, 2015). All the analyses were performed separately for short-term and longer-term sampling periods. Response data were transformed by taking the natural logarithm of each value when needed to meet the assumptions of normality and homogeneity of variance.

Results

Short-term and longer-term soil respiration and Q_{10} responses to warming

Warming significantly increased soil respiration during the first two years of the experiment in the high biocrust cover plots (Figure 1a, Table S1, $p = 0.029$). However, these positive effects disappeared in the longer-term (i.e. 8 to 10 years after experimental setup; Figure 1b, Table S3, $p=0.457$). Seasonally, soil respiration rates were consistently greater in autumn and spring, matching major precipitation events over both the short- (Figure 2a) and the longer-term (Figure 2b). The Q_{10} was similar in warmed and control plots in the short-term (Figure 1c, $p = 0.818$), but this variable was a 10% lower (95% CI= 9 to 11%) in warmed than in control plots for both biocrust cover levels in the longer-term (Figure 1d, $p < 0.001$). The effects of warming and biocrust cover level on soil respiration and Q_{10} at both sampling periods were similar to those addressed when using the original data without imputation (Table S2 and S4, Fig. S3).

Changes in soil microclimatic variables as a driver of soil respiration responses to warming

On average, soil temperature was 2.95°C (95% CI= 2.90 to 2.99 °C) and 1.43°C (95% CI= 1.39 to 1.48 °C) higher in warmed than in control plots at both short and longer-term periods, respectively (Figure S4a and b, respectively, $p < 0.001$ for both periods).

Seasonally, differences in soil temperature between control and warmed plots were greater in summer (i.e. from July to September) both in the short- (Figure 2c) and longer-term (Figure 2d).

On the contrary, differences in soil moisture measured monthly between control and warming plots were higher in winter (i.e. from December to March) both in the short- (Figure 2e) and longer-term (Figure 2f). These results can be also observed when using continuous soil moisture measurements (Figure S5a and S5b for short- and longer-term, respectively). Indeed, soil moisture showed a similar pattern independently of the measurement frequency (i.e. monthly or continuous)

both in the short- (Figure S5c and S5e, respectively) and in the longer-term (Figure S5d and S5f, respectively).

When evaluating the effect of warming on soil moisture measured monthly in the short-term, we found that this variable was 1.5% (95% CI= 0.97 to 1.55%) lower in warmed than in control plots (Figure S6a, $p=0.005$); significant warming effects were not observed when using continuous soil moisture data (1.6% reduction; Figure S6a $p=0.851$). We did not find significant differences in soil moisture between control and warming plots in the longer-term, neither when using monthly nor continuous soil moisture data (Figure S6b, $p=0.696$ and Figure S6b, $p=0.163$, respectively).

The effect size of warming on soil respiration, as measured with the response ratio, increased when the warming effect on soil temperature was higher under short-term warming (Figure 3a). Contrarily, the effect sizes of warming on soil respiration and soil temperature were not related under longer-term warming (Figure 3b). On the other hand, the effect sizes of warming on soil respiration and moisture were not related in the short- (Figure 3c) and longer-term (Figure 3d) periods.

Changes in biocrust cover as a driver of soil respiration responses to warming

In the short-term, the total biocrust cover was similar in warmed (9.40%, 95% CI= 8.84 to 9.96% and 66.27%, 95% CI= 63.80 to 68.80%, for low and high initial biocrust cover respectively) and control (7.94%, 95% CI= 7.51 to 8.37% and 64.18%, 95%CI=62.43 to 65.92%, for low and high initial biocrust cover respectively) plots (Figure S7a, $p=0.737$). In the longer-term, this pattern changed dramatically (Figure S7b), as warming significantly ($p<0.001$) decreased total biocrust cover by 26.78% (95% CI= 25.85 to 27.70%) in plots with low initial biocrust cover and by 27.50% (95% CI= 27.17 to 27.83%) in plots with high biocrust cover. The effect size of warming on total biocrust cover and soil respiration were unrelated in the short-term (Figure 4a). However, these effect sizes were significantly and positively related in the longer-term (Figure 4b), indicating that decreases in biocrust cover with warming matched with a reduction in soil respiration.

Microbial thermal acclimation as a driver of longer-term soil respiration responses to field warming

We did not find any significant differences in soil microbial biomass between warming and control plots independently of the method used to estimate it (Figure S8; $p=0.813$, $p=0.810$ and $p=0.712$ for the Yeast-SIR, CFE and qPCR method, respectively).

Although the positive effects of assay temperature on potential soil microbial respiration rates were the largest in magnitude by far (Figure 5, Table S5, $p < 0.001$), we also found a negative effect of the warming treatment on soil microbial respiration (Figure 5, Table S5, $p = 0.002$). This effect was, on average, a 30% lower across all assay temperatures. Importantly, this reduction accounted for potential differences in soil microbial biomass (models statistically controlled for differences in microbial biomass), and substrate limitation (incubations were performed with substrate in excess), and were observed independently of the method used to estimate microbial biomass (Table S5).

Discussion

The positive effect of warming on soil respiration observed in the short-term in plots with high initial biocrust cover disappeared after ten years of experimental warming. This longer-term response to warming was linked to a decrease in Q_{10} in the warmed compared to the control plots. Additionally, we found support for several mechanisms driving short and longer-term soil respiration responses to warming such as warming-induced increases in soil temperature, microbial thermal acclimation and changes in total biocrust cover. These mechanisms are discussed in detail below.

Short-term studies have found contrasting soil respiration responses to warming in drylands, ranging from positive (Darrouzet-Nardi et al., 2015; Shen, Reynolds, & Hui, 2009) to negative (García-Palacios et al., 2018; Xu, Hou, Zhang, Liu, & Zhou, 2016). The rare dryland studies that have evaluated warming effects for more than five years have found that soil respiration rates return to control levels after few years of warming (Darrouzet-Nardi et al., 2018; García-Palacios et al., 2018). We compared soil respiration responses to warming in the short- vs. longer-term and found a positive warming effect in areas with high initial biocrust cover after two years of warming. This positive short-term effect was not sustained through time, and it disappeared after ten years of elevated temperatures. Accordingly, Q_{10} values were significantly lower in the warmed plots compared to the control plots under longer-term warming. The mismatch between our short-term results and previous studies also conducted in drylands (García-Palacios et al., 2018; Xu et al., 2016) may be caused by different soil respiration responses to

warming due to changes in the mechanisms driving such responses. Such changes in the mechanisms underlying soil respiration responses to warming may also explain the differences between the warming effects on soil respiration observed short- and longer-term in our study. Therefore, to better understand soil respiration responses to warming both in the short and longer-term, the different drivers that could regulate such responses must be investigated.

Warming-induced increases in soil temperature led to a rise in soil respiration in the short-term, especially in areas with high biocrust cover. Such warming effects on soil respiration may be influenced by significant peaks of soil respiration after small rainfall or dew events in the study area (Cable & Huxman, 2004; Ladrón de Guevara et al., 2014). For instance, peaks in soil respiration have been observed in a biocrusted site in the Kalahari Sands (Botswana) after rainfall events of just 1.6 mm (Thomas, Hoon, & Dougill, 2011). Therefore, increases in soil temperature were the main driver underlying the short-term soil respiration responses to warming, given that the mean soil moisture observed (8.5% in the short-term) may be enough to support microbial activity. However, we did not observe this direct effect of warming-induced elevated temperature on soil respiration in the longer-term. The disagreement between this result and the expectation that soil respiration should increase with warming (Kirschbaum, 2006) may be a consequence of a longer-term effect of the warming treatment on the biocrust and soil microbial communities, compensating the direct effect of increased temperatures.

Experimental warming reduced soil moisture when measured monthly by 1.5% in the short-term, whereas it did not have any effect in the longer-term (0.2% reduction). However, no differences in soil moisture were observed when it was continuously measured neither in the short- nor in the longer-term. The difference in the results observed between both soil moisture sampling frequencies may be due to singularities of a specific sampling date, given that there were no differences in soil moisture measured continuously between warming and control plots during long periods both in the short- and the longer-term. Additionally, a similar pattern was observed independently of the measurement frequency, suggesting that both methods are appropriate to measure soil moisture to test its effect on soil respiration responses to warming. This absence of differences in soil moisture during long periods both in the short- and longer-term may explain why the soil respiration responses to warming were independent of changes in soil moisture over both periods. Our results indicate that soil respiration responses to warming are not a product of a reduction in soil microbial activity with warming-induced soil drying, which disagrees with the results found in previous dryland studies (Pendall et al., 2013; Rey et al., 2011). This mismatch

between our results and previous findings may be a consequence of the magnitude of the soil moisture change induced by warming. For instance, in Pendall et al., (2013) soil respiration responses to warming were mediated by a 15% decrease in soil moisture. Therefore, the warming-induced reduction of soil moisture observed in our study may not be large enough to drive soil respiration responses to warming. Additionally, soil respiration in drylands is not only controlled by rainfall events but also by dew generated in the early morning (Rey et al., 2011), as dew-like water inputs were enough to stimulate the respiration of biocrust-forming lichens and the soil microorganisms associated to them (Delgado-Baquerizo, Maestre, Rodríguez, & Gallardo, 2013; Ladrón de Guevara et al., 2014). Therefore, the increased activity of biocrusts, which are a major contributor to soil respiration in our study area, due to water inputs derived from dew events may explain why soil moisture was not driving soil respiration responses to warming either in the short- or in the longer-term. For all these reasons, we acknowledge how a more detailed temporal evaluation of air and soil moisture and temperature data (as well as dew events) will be needed in future studies to help disentangle the abiotic component of warming effects on soil respiration. According to our soil temperature and moisture results, warming-induced changes in soil microclimatic variables do not seem to be the main mechanism underlying observed soil respiration responses to elevated temperatures in the longer-term. Therefore, we tested whether changes in biocrust cover and thermal acclimation of soil microbiota could be driving such responses.

We observed that soil respiration was larger in the plots with high initial biocrust cover (compared with those with a low initial biocrust cover) during both warming periods, albeit temporal trends of soil respiration were similar at both biocrust cover levels. These results agree with those observed in previous studies showing greater respiration rates in areas with visible and well-developed biocrusts (Castillo-Monroy, Maestre, et al., 2011; Feng et al., 2013). Accordingly, higher soil respiration rates have also found in areas with lichen-dominated biocrusts than in those dominated by mosses or algae (Feng et al., 2014). Therefore, the differences in soil respiration between low and high biocrust cover plots observed in our study may be a result of the biological activity of the mosses and lichens directly or through their effect on soil microbial communities (Castillo-Monroy, Bowker, et al., 2011). On the other hand, our results showed that soil respiration responses to short-term warming were independent of changes in biocrust cover, as biocrusts were not affected by the warming treatment in the short-term. Contrary, we observed that soil respiration responses to warming were mediated by warming-induced reductions in biocrust cover

in the longer-term. The observed decrease in biocrust cover with warming may not seem consistent with previous findings showing that lichens are well adapted to elevated temperatures and are resistant to desiccation (Green, Sancho, & Pintado, 2011). However, it agrees with other studies conducted in drylands which found an important reduction in biocrust cover after some years of warming (Ferrenberg et al., 2015; Maestre, Escolar, et al., 2015). Although clarifying the physiological mechanisms underlying this dramatic decrease in biocrust cover under longer-term warming is not a goal of this study, we speculate that warming effects led to a significant reduction of C fixation and subsequent mortality of lichens (Ladrón de Guevara et al., 2014). This biocrust cover reduction would, therefore, lower the autotrophic soil respiration that, may explain the decreased soil respiration rates observed in the warmed plots in the longer-term. To sum up, our results suggest that biocrusts modulate soil respiration rates and that warming-induced changes in their cover are one of the main drivers governing observing soil respiration responses to longer-term warming.

Finally, we found a negative effect of field warming on soil microbial respiration at a common biomass and excess substrate in the laboratory incubations. This result highlights that soil microbial respiration acclimated to warming conditions in the dryland ecosystem studied and suggests that thermal acclimation may drive the lack of warming effects on soil respiration over the longer-term. Importantly, this result was observed regardless of the method used to measure microbial biomass, suggesting that substrate-induced respiration is an appropriate estimate of microbial biomass to test for thermal acclimation of soil respiration (Bradford et al., 2008, 2009). The negative field warming effect on soil microbial respiration observed is consistent with biochemical acclimation to different thermal regimes reached through evolutionary trade-offs (Hochachka & Somero, 2002). However, we cannot state that biochemical acclimation is the only mechanism operating to explain our results as they may be caused by shifts at the individual, population and community levels due to the 'aggregate' respiratory activity of soil microbial communities and the spatial scale we were analysing. Although the observed negative effect of warming on potential soil microbial respiration rates may seem incompatible with the expected positive link between temperature and soil microbial respiration rates (Davidson & Janssens, 2006; Kirschbaum, 2006; Lloyd & Taylor, 1994; Tucker et al., 2013), such positive relationship was supported by the conspicuous positive effect of assay temperature on respiration rates observed in our study. The thermal acclimation of soil respiration observed in this study provides empirical support to previous global extrapolations showing that soil C losses to the atmosphere

via soil respiration with elevated temperature may be lower in drylands than in other biomes (Crowther et al., 2016). Indeed, in a global study analysing data from 27 different temperature manipulation experiments, spanning nine biomes, drylands and boreal are the only ecosystems where differences in temperature sensitivity between warmed and control plots have been found (Carey et al., 2016). Therefore, they only found evidence for thermal acclimation of soil respiration in drylands and boreal forests, agreeing with our results.

In conclusion, we found that short-term increases in soil respiration with warming disappeared after ten years of continuous warming in a biocrust-dominated dryland. This pattern was associated with a longer-term decrease in temperature sensitivity of soil respiration (Q_{10}). Our results suggest that the main driver regulating short-term soil respiration responses to warming was the increase in soil temperature, whereas both thermal acclimation and a dramatic loss of biocrust cover drove soil respiration responses to warming in the longer-term. Our results highlight the need to evaluate the effects of warming at both the short- and longer-term to better understand soil respiration responses to this climate change driver and the important role that longer-term experiments play for doing so. They also emphasize the need to include both thermal acclimation and biocrust communities in models aiming to forecast soil greenhouse gas emission predictions in drylands, as this would improve our capacity to forecast future temperatures and expand our understanding of C - climate feedbacks.

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Competing interests

The authors declare no competing financial interests.

Author

F.T.M. designed the field study and wrote the grant that funded the work. F.T.M, P.G.P and M.D. developed the original idea of the analyses presented in the manuscript. M.D. performed the statistical analyses, with inputs from F.T.M and P.G.P. M. D., S.A., C.C.-D., B.G. and V. O. conducted the field and laboratory work. All authors contributed to data interpretation. M.D. wrote the first version of the manuscript, which was revised by all co-authors.

Data Sharing and Data Accessibility

The data that support the findings of this study and the R code are openly available in Figshare at <http://doi.org/10.6084/m9.figshare.11536989>.

References

- Atkin, O. K., & Tjoelker, M. G. (2003). Thermal acclimation and the dynamic response of plant respiration to temperature. *Trends in Plant Science*, 8(7), 343–351.
[https://doi.org/10.1016/S1360-1385\(03\)00136-5](https://doi.org/10.1016/S1360-1385(03)00136-5)
- Bates, D., Mächler, M., Bolker, B. M., & Walker, S. C. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67(1), 1–48.
<https://doi.org/10.18637/jss.v067.i01>
- Berdugo, M., Soliveres, S., & Maestre, F. T. (2014). Vascular plants and biocrusts modulate how abiotic factors affect wetting and drying events in drylands. *Ecosystems*, 17(7), 1242–1256.
<https://doi.org/10.1007/s10021-014-9790-4>
- Bradford, M. A., Davies, C. A., Frey, S. D., Maddox, T. R., Melillo, J. M., Mohan, J. E., ... Wallenstein, M. D. (2008). Thermal adaptation of soil microbial respiration to elevated temperature. *Ecology Letters*, 11(12), 1316–1327. <https://doi.org/10.1111/j.1461-0248.2008.01251.x>
- Bradford, M. A., McCulley, R. L., Crowther, T. W., Oldfield, E. E., Wood, S. A., & Fierer, N. (2019). Cross-biome patterns in soil microbial respiration predictable from evolutionary theory on thermal adaptation. *Nature Ecology & Evolution*, 3(2), 223–231.
<https://doi.org/10.1038/s41559-018-0771-4>
- Bradford, M. A., Wallenstein, M. D., Allison, S. D., Treseder, K. K., Frey, S. D., Watts, B. W., ... Reynolds, J. F. (2009). Decreased mass specific respiration under experimental warming is robust to the microbial biomass method employed. *Ecology Letters*, 12(7), 18–21.
<https://doi.org/10.1111/j.1461-0248.2009.01332.x>

Bradford, M. A., Watts, B. W., & Davies, C. A. (2010). Thermal adaptation of heterotrophic soil respiration in laboratory microcosms. *Global Change Biology*, 16(5), 1576–1588.

<https://doi.org/10.1111/j.1365-2486.2009.02040.x>

Bradford, M. A., Wieder, W. R., Bonan, G. B., Fierer, N., Raymond, P. A., & Crowther, T. W. (2016). Managing uncertainty in soil carbon feedbacks to climate change. *Nature Climate Change*, 6(8), 751–758. <https://doi.org/10.1038/nclimate3071>

Breiman, L. (2001). Random forests. *Machine Learning*, 45(1), 5–32.

<https://doi.org/10.1023/A:1010933404324>

Cable, J. M., & Huxman, T. E. (2004). Precipitation pulse size effects on Sonoran Desert soil microbial crusts. *Oecologia*, 141(2), 317–324. <https://doi.org/10.1007/s00442-003-1461-7>

Campbell, C. D., Chapman, S. J., Cameron, C. M., Davidson, M. S., & Potts, J. M. (2003). A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. *Applied and Environmental Microbiology*, 69(6), 3593–3599.

<https://doi.org/10.1128/AEM.69.6.3593>

Cano-Díaz, C., Mateo, P., Muñoz-Martín, M. Á., & Maestre, F. T. (2018). Diversity of biocrust-forming cyanobacteria in a semiarid gypsiferous site from Central Spain. *Journal of Arid Environments*, 151(October 2017), 83–89. <https://doi.org/10.1016/j.jaridenv.2017.11.008>

Carey, J. C., Tang, J., Templer, P. H., Kroeger, K. D., Crowther, T. W., Burton, A. J., ... Tietema, A. (2016). Temperature response of soil respiration largely unaltered with experimental warming. *Proceedings of the National Academy of Sciences*, 113(48), 13797–13802.

<https://doi.org/10.1073/pnas.1605365113>

Castillo-Monroy, A. P., Bowker, M. A., Maestre, F. T., Rodríguez-Echeverría, S., Martinez, I., Barraza-Zepeda, C. E., & Escolar, C. (2011). Relationships between biological soil crusts, bacterial diversity and abundance, and ecosystem functioning: Insights from a semi-arid Mediterranean environment. *Journal of Vegetation Science*, 22(1), 165–174.

<https://doi.org/10.1111/j.1654-1103.2010.01236.x>

Castillo-Monroy, A. P., Maestre, F. T., Rey, A., Soliveres, S., & García-Palacios, P. (2011). Biological soil crust microsites are the main contributor to soil respiration in a semiarid ecosystem. *Ecosystems*, 14(5), 835–847. <https://doi.org/10.1007/s10021-011-9449-3>

Cherlet, M., Hutchinson, C., Reynolds, J., Hill, J., Sommer, S., & Maltitz, G. (2018). *World Atlas of Desertification*. Publication Office of the European Union, Luxembourg.

Ciais, P., Sabine, C., Bala, G., Bopp, L., Brovkin, V., Canadell, A., ... Thornton, P. (2014).

Carbon and Other Biogeochemical Cycles. In *Climate Change (2013): The physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the intergovernmental Panel on Climate Change* (pp. 465–570). Cambridge University Press.

Conant, R. T., Dalla-Betta, P., Klopatek, C. C., & Klopatek, J. M. (2004). Controls on soil respiration in semiarid soils. *Soil Biology and Biochemistry*, 36(6), 945–951.

<https://doi.org/10.1016/j.soilbio.2004.02.013>

Crowther, T., Todd-Brown, K., Rowe, C., Wieder, W., Carey, J., Machmuller, M., ... Bradford, M. (2016). Quantifying global soil C losses in response to warming. *Nature*, 540, 104–108.

<https://doi.org/10.1038/nature20150>

Dacal, M., Bradford, M. A., Plaza, C., Maestre, F. T., & García-Palacios, P. (2019). Soil microbial respiration adapts to ambient temperature in global drylands. *Nature Ecology & Evolution*, 3(2), 232–238. <https://doi.org/10.1038/s41559-018-0770-5>

Darrouzet-Nardi, A., Reed, S. C., Grote, E. E., & Belnap, J. (2015). Observations of net soil exchange of CO₂ in a dryland show experimental warming increases carbon losses in biocrust soils. *Biogeochemistry*, 126(3), 363–378. <https://doi.org/10.1007/s10533-015-0163-7>

Darrouzet-Nardi, A., Reed, S. C., Grote, E. E., & Belnap, J. (2018). Patterns of longer-term climate change effects on CO₂ efflux from biocrusted soils differ from those observed in the short term. *Biogeosciences*, 15(14), 4561–4573. <https://doi.org/10.5194/bg-15-4561-2018>

Davidson, E. A., & Janssens, I. A. (2006). Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature*, 440. <https://doi.org/10.1038/nature04514>

Davidson, E. A., Janssens, I. A., & Luo, Y. (2006). On the variability of respiration in terrestrial ecosystems : moving beyond Q₁₀. *Global Change Biology*, 12, 154–164.

<https://doi.org/10.1111/j.1365-2486.2005.01065.x>

De Castro, M., Martín-Vide, J., & Alonso, S. (2005). El clima de España: pasado, presente y escenarios de clima para el siglo XXI. In *Evaluación preliminar de los impactos en España por efecto del cambio climático* (pp. 1–64). Madrid, Spain: Ministerio Medio Ambiente.

del Prado, R., & Sancho, L. G. (2007). Dew as a key factor for the distribution pattern of the lichen species *Teloschistes lacunosus* in the Tabernas Desert (Spain). *Flora: Morphology, Distribution, Functional Ecology of Plants*, 202(5), 417–428.

<https://doi.org/10.1016/j.flora.2006.07.007>

Delgado-Baquerizo, M., Maestre, F. T., Rodríguez, J. G. P., & Gallardo, A. (2013). Biological soil

- crusts promote N accumulation in response to dew events in dryland soils. *Soil Biology and Biochemistry*, 62, 22–27. <https://doi.org/10.1016/j.soilbio.2013.02.015>
- Elbert, W., Weber, B., Burrows, S., Steinkamp, J., Büdel, B., Andreae, M. O., & Pöschl, U. (2012). Contribution of cryptogamic covers to the global cycles of carbon and nitrogen. *Nature Geoscience*, 5(7), 459–462. <https://doi.org/10.1038/ngeo1486>
- Escolar, C., Maestre, F. T., & Rey, A. (2015). Biocrusts modulate warming and rainfall exclusion effects on soil respiration in a semi-arid grassland. *Soil Biology and Biochemistry*, 80, 9–17. <https://doi.org/10.1016/j.soilbio.2014.09.019>
- Escolar, C., Martínez, I., Bowker, M. A., & Maestre, F. T. (2012). Warming reduces the growth and diversity of biological soil crusts in a semi-arid environment: Implications for ecosystem structure and functioning. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 367(1606), 3087–3099. <https://doi.org/10.1098/rstb.2011.0344>
- Feng, W., Zhang, Y., Jia, X., Wu, B., Zha, T., Qin, S., ... Fa, K. (2014). Impact of environmental factors and biological soil crust types on soil respiration in a desert ecosystem. *PLoS ONE*, 9(7). <https://doi.org/10.1371/journal.pone.0102954>
- Feng, W., Zhang, Y. Q., Wu, B., Zha, T. S., Jia, X., Qin, S. G., ... Fa, K. Y. (2013). Influence of disturbance on soil respiration in biologically crusted soil during the dry season. *The Scientific World Journal*, 2013. <https://doi.org/10.1155/2013/408560>
- Ferrenberg, S., Reed, S. C., Belnap, J., & Schlesinger, W. H. (2015). Climate change and physical disturbance cause similar community shifts in biological soil crusts. *Proceedings of the National Academy of Sciences of the United States of America*, 112(39), 12116–12121. <https://doi.org/10.1073/pnas.1509150112>
- Fierer, N., Schimel, J. P., & Holden, P. A. (2003). Variations in microbial community composition through two soil depth profiles. *Soil Biology and Biochemistry*, 35, 167–176.
- García-Palacios, P., Escolar, C., Dacal, M., Delgado-Baquerizo, M., Gozalo, B., Ochoa, V., & Maestre, F. T. (2018). Pathways regulating decreased soil respiration with warming in a biocrust-dominated dryland. *Global Change Biology*, 24(10), 4645–4656. <https://doi.org/10.1111/gcb.14399>
- Green, T. G. A., Sancho, L. G., & Pintado, A. (2011). Ecophysiology of desiccation/rehydration cycles in mosses and lichens. In U. Lüttge, E. Beck, & D. Bartels (Eds.), *Plant desiccation tolerance* (pp. 89–120). Springer Berlin Heidelberg. <https://doi.org/https://doi.org/10.1007/978-3-642-19106-0>

- Grote, E. E., Belnap, J., Housman, D. C., & Sparks, J. P. (2010). Carbon exchange in biological soil crust communities under differential temperatures and soil water contents: implications for global change. *Global Change Biology*, 16(10), 2763–2774.
<https://doi.org/10.1111/j.1365-2486.2010.02201.x>
- Guan, C., Li, X., Zhang, P., & Li, C. (2019). Effect of global warming on soil respiration and cumulative carbon release in biocrust-dominated areas in the Tengger Desert, northern China. *Journal of Soils and Sediments*, 19(3), 1161–1170. <https://doi.org/10.1007/s11368-018-2171-4>
- Hartley, I. P., Hopkins, D. W., Garnett, M. H., Sommerkorn, M., & Wookey, P. A. (2008). Soil microbial respiration in arctic soil does not acclimate to temperature. *Ecology Letters*, 11(10), 1092–1100. <https://doi.org/10.1111/j.1461-0248.2008.01223.x>
- Hartley, I. P., Hopkins, D. W., Garnett, M. H., Sommerkorn, M., & Wookey, P. A. (2009). No evidence for compensatory thermal adaptation of soil microbial respiration in the study of Bradford et al. (2008). *Ecology Letters*, 12(7), 14–16. <https://doi.org/10.1111/j.1461-0248.2009.01300.x>
- Hedges, L. V., Gurevitch, J., & Curtis, P. S. (1999). The meta-analysis of response ratios in experimental ecology. *Ecology*, 80(4), 1150–1156. [https://doi.org/10.1890/0012-9658\(1999\)080\[1150:TMAORR\]2.0.CO;2](https://doi.org/10.1890/0012-9658(1999)080[1150:TMAORR]2.0.CO;2)
- Hochachka, P. W., & Somero, G. N. (2002). *Biochemical adaptation: mechanism and process in physiological evolution*. New York: Oxford University Press.
- IUSS Working Group WRB. (2006). *World reference base for soil resources 2006. World Soil Resources Reports No. 103. Encyclopedia of Environmental Change*. Rome, Italy: FAO.
<https://doi.org/10.4135/9781446247501.n4190>
- Jasienski, M., & Bazzaz, F. (1999). The fallacy of ratios and the testability of models in biology. *Oikos*, 84(2), 321–326.
- Kirschbaum, M. U. F. (2004). Soil respiration under prolonged soil warming: Are rate reductions caused by acclimation or substrate loss? *Global Change Biology*, 10(11), 1870–1877.
<https://doi.org/10.1111/j.1365-2486.2004.00852.x>
- Kirschbaum, M. U. F. (2006). The temperature dependence of organic-matter decomposition — still a topic of debate. *Soil Biology and Biochemistry*, 38, 2510–2518.
<https://doi.org/10.1016/j.soilbio.2006.01.030>
- Ladrón de Guevara, M., Gozalo, B., Raggio, J., Lafuente, A., Prieto, M., & Maestre, F. T. (2018).

Warming reduces the cover, richness and evenness of lichen-dominated biocrusts but promotes moss growth: insights from an 8 yr experiment. *New Phytologist*, 220(3), 811–823. <https://doi.org/10.1111/nph.15000>

Ladrón de Guevara, M., Lázaro, R., Quero, J. L., Ochoa, V., Gozalo, B., Berdugo, M., ... Maestre, F. T. (2014). Simulated climate change reduced the capacity of lichen-dominated biocrusts to act as carbon sinks in two semi-arid Mediterranean ecosystems. *Biodiversity and Conservation*, 23(7), 1787–1807. <https://doi.org/10.1007/s10531-014-0681-y>

Lafuente, A., Berdugo, M., Ladrón de Guevara, M., Gozalo, B., & Maestre, F. T. (2018). Simulated climate change affects how biocrusts modulate water gains and desiccation dynamics after rainfall events. *Ecohydrology*, 11(6). <https://doi.org/10.1002/eco.1935>

Lloyd, J., & Taylor, J. A. (1994). On the temperature dependence of soil respiration. *Functional Ecology*, 8(3), 315–323.

Lundegardh, H. (1927). Carbon dioxide evolution of soil and crop growth. *Soil Science*, 23(6), 415–453.

Luo, Y., Wan, S., Hui, D., & Wallace, L. L. (2001). Acclimatization of soil respiration to warming in a tall grass prairie. *Nature*, 413(October), 622–625. <https://doi.org/10.1038/35098065>

Luo, Y., & Zhou, X. (2006). *Soil Respiration and the Environment*. Burlington, MA, USA: Academic Press.

Maestre, F. T., Delgado-Baquerizo, M., Jeffries, T. C., Eldridge, D. J., & Ochoa, V. (2015). Increasing aridity reduces soil microbial diversity and abundance in global drylands. *Proceedings of the National Academy of Sciences*, 112(51), 15684–15689. <https://doi.org/10.1073/pnas.1516684112>

Maestre, F. T., Escolar, C., Bardgett, R. D., Dungait, J. A. J., Gozalo, B., & Ochoa, V. (2015). Warming reduces the cover and diversity of biocrust-forming mosses and lichens, and increases the physiological stress of soil microbial communities in a semi-arid *Pinus halepensis* plantation. *Frontiers in Microbiology*, 6(AUG), 1–12. <https://doi.org/10.3389/fmicb.2015.00865>

Maestre, F. T., Escolar, C., de Guevara, M. L., Quero, J. L., Lázaro, R., Delgado-Baquerizo, M., ... Gallardo, A. (2013). Changes in biocrust cover drive carbon cycle responses to climate change in drylands. *Global Change Biology*, 19(12), 3835–3847. <https://doi.org/10.1111/gcb.12306>

Maphangwa, K. W., Musil, C. F., Raitt, L., & Zedda, L. (2012). Experimental climate warming

decreases photosynthetic efficiency of lichens in an arid South African ecosystem.

Oecologia, 169(1), 257–268. <https://doi.org/10.1007/s00442-011-2184-9>

Melillo, J. M., Frey, S. D., DeAngelis, K. M., Werner, W. J., Bernard, M. J., Bowles, F. P., ...

Grandy, A. S. (2017). Long-term pattern and magnitude of soil carbon feedback to the climate system in a warming world. *Science*, 358(6359), 101–105.

<https://doi.org/10.1126/science.aan2874>

Melillo, J. M., Steudler, P. A., Aber, J. D., Newkirk, K., Lux, H., Bowles, F. P., ... Morrisseau, S. (2002). Soil warming and carbon-cycle feedbacks to the climate system. *Science*, 298, 2173–2176. <https://doi.org/10.1126/science.1074153>

Ouyang, H., & Hu, C. (2017). Insight into climate change from the carbon exchange of biocrusts utilizing non-rainfall water. *Scientific Reports*, 7(1), 1–13. <https://doi.org/10.1038/s41598-017-02812-y>

Pendall, E., Heisler-White, J. L., Williams, D. G., Dijkstra, F. A., Carrillo, Y., Morgan, J. A., & LeCain, D. R. (2013). Warming Reduces Carbon Losses from Grassland Exposed to Elevated Atmospheric Carbon Dioxide. *PLoS ONE*, 8(8), e71921.

<https://doi.org/10.1371/journal.pone.0071921>

Plaza, C., Zaccone, C., Sawicka, K., Méndez, A. M., Tarquis, A., Gascó, G., ... Maestre, F. T. (2018). Soil resources and element stocks in drylands to face global issues. *Scientific Reports*, 8(1), 13788. <https://doi.org/10.1038/s41598-018-32229-0>

R Core Team. (2015). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from <http://www.r-project.org/>

Rey, A., Pegoraro, E., Oyonarte, C., Were, A., Escibano, P., & Raimundo, J. (2011). Impact of land degradation on soil respiration in a steppe (*Stipa tenacissima* L .) semi-arid ecosystem in the SE of Spain. *Soil Biology and Biochemistry*, 43(2), 393–403.

<https://doi.org/10.1016/j.soilbio.2010.11.007>

Rodriguez-Caballero, E., Belnap, J., Büdel, B., Crutzen, P. J., Andreae, M. O., Pöschl, U., & Weber, B. (2018). Dryland photoautotrophic soil surface communities endangered by global change. *Nature Geoscience*, 11(3), 185–189. <https://doi.org/10.1038/s41561-018-0072-1>

Schindlbacher, A., Schneck, J., Takriti, M., Borken, W., & Wanek, W. (2015). Microbial physiology and soil CO₂ efflux after 9 years of soil warming in a temperate forest - no indications for thermal adaptations. *Global Change Biology*, 21(11), 4265–4277.

<https://doi.org/10.1111/gcb.12996>

- Shen, W., Reynolds, J. F., & Hui, D. (2009). Responses of dryland soil respiration and soil carbon pool size to abrupt vs. gradual and individual vs. combined changes in soil temperature, precipitation, and atmospheric [CO₂]: a simulation analysis. *Global Change Biology*, 15(9), 2274–2294. <https://doi.org/10.1111/j.1365-2486.2009.01857.x>
- Stekhoven, D. J., & Bühlmann, P. (2012). Missforest-Non-parametric missing value imputation for mixed-type data. *Bioinformatics*, 28(1), 112–118. <https://doi.org/10.1093/bioinformatics/btr597>
- Thomas, A. D., Hoon, S. R., & Dougill, A. J. (2011). Soil respiration at five sites along the Kalahari transect: effects of temperature, precipitation pulses and biological soil crust cover. *Geoderma*, 167–168, 284–294. <https://doi.org/10.1016/j.geoderma.2011.07.034>
- Topp, G. C., & Davis, J. L. (1985). Measurement of Soil Water Content using Time-domain Reflectometry (TDR): A Field Evaluation1. *Soil Science Society of America Journal*, 49(1), 19. <https://doi.org/10.2136/sssaj1985.03615995004900010003x>
- Tucker, C. L., Bell, J., Pendall, E., & Ogle, K. (2013). Does declining carbon-use efficiency explain thermal acclimation of soil respiration with warming? *Global Change Biology*, 19(1), 252–263. <https://doi.org/10.1111/gcb.12036>
- Vance, E. D., Brookes, P. C., & Jenkinson, D. S. (1987). An extraction method for measuring microbial biomass C. *Soil Biology and Biochemistry*, 19(6), 703–707.
- Veste, M., Littmann, T., Friedrich, H., & Breckle, S. W. (2001). Microclimatic boundary conditions for activity of soil lichen crusts in sand dunes of the north-western Negev desert, Israel. *Flora*, 196(6), 465–474. [https://doi.org/10.1016/S0367-2530\(17\)30088-9](https://doi.org/10.1016/S0367-2530(17)30088-9)
- Wang, X., Liu, L., Piao, S., Janssens, I. A., Tang, J., Liu, W., ... Xu, S. (2014). Soil respiration under climate warming: Differential response of heterotrophic and autotrophic respiration. *Global Change Biology*, 20(10), 3229–3237. <https://doi.org/10.1111/gcb.12620>
- Weber, B., Büdel, B., & Belnap, J. (Eds.). (2016). *Biological soil crusts: an organizing principle in drylands* (Vol. 226). Springer International Publishing. <https://doi.org/10.1007/978-3-319-30214-0>
- Xu, Z., Hou, Y., Zhang, L., Liu, T., & Zhou, G. (2016). Ecosystem responses to warming and watering in typical and desert steppes. *Scientific Reports*, 6(October), 1–14. <https://doi.org/10.1038/srep34801>
- Zhou, J., Xue, K., Xie, J., Deng, Y., Wu, L., Cheng, X., ... Luo, Y. (2012). Microbial mediation of carbon-cycle feedbacks to climate warming. *Nature Climate Change*, 2(2), 106–110. <https://doi.org/10.1038/nclimate1331>

Figure legends:

Figure 1. Warming effects on soil respiration rates (a-b) and the temperature sensitivity of soil respiration (Q_{10} , c-d) in the short-term (0 – 2 years after experimental set-up, a and c) and longer-term (8- 10 years after experimental set-up, b and d) at both biocrust cover levels (i.e. low and high). Box plots represent medians, 25th and 75th percentiles ($n=210$ and 240 per combination of treatments at the short and longer-term, respectively for soil respiration measurements and $n = 10$ per combination of treatments at each period for Q_{10}). Error bars represent 10th and 90th percentiles. Asterisks denote significant differences at $p < 0.05$. These analyses were performed with an 11% data imputation. The same results performed using original data without imputing missing values are shown in Fig. S3.

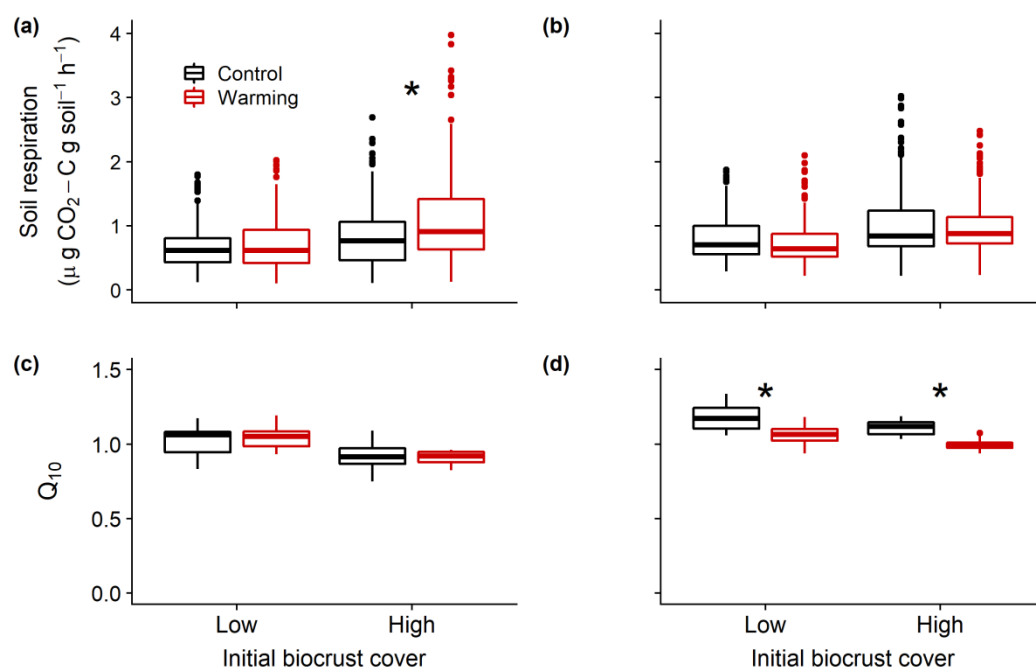
Figure 2. Soil respiration, temperature and moisture measured across short- (after experimental set-up, a, c, e) and longer-term (8 – 10 years after experimental set-up, b, d, f) periods. Data are means \pm SE ($n=10$). WA = warming. Low and high refers to initial biocrust cover $< 20\%$ and $> 50\%$, respectively.

Figure 3. Relationship between the effect size of warming (RR) on soil respiration and soil temperature in the short- (a) and longer-term (b), and between RR of soil respiration and RR of soil moisture in the short- (c) and longer-term (d). RR data are in ln- scale. The solid line denotes a significant linear model fitted between both variables. (R^2 and p values on the graph).

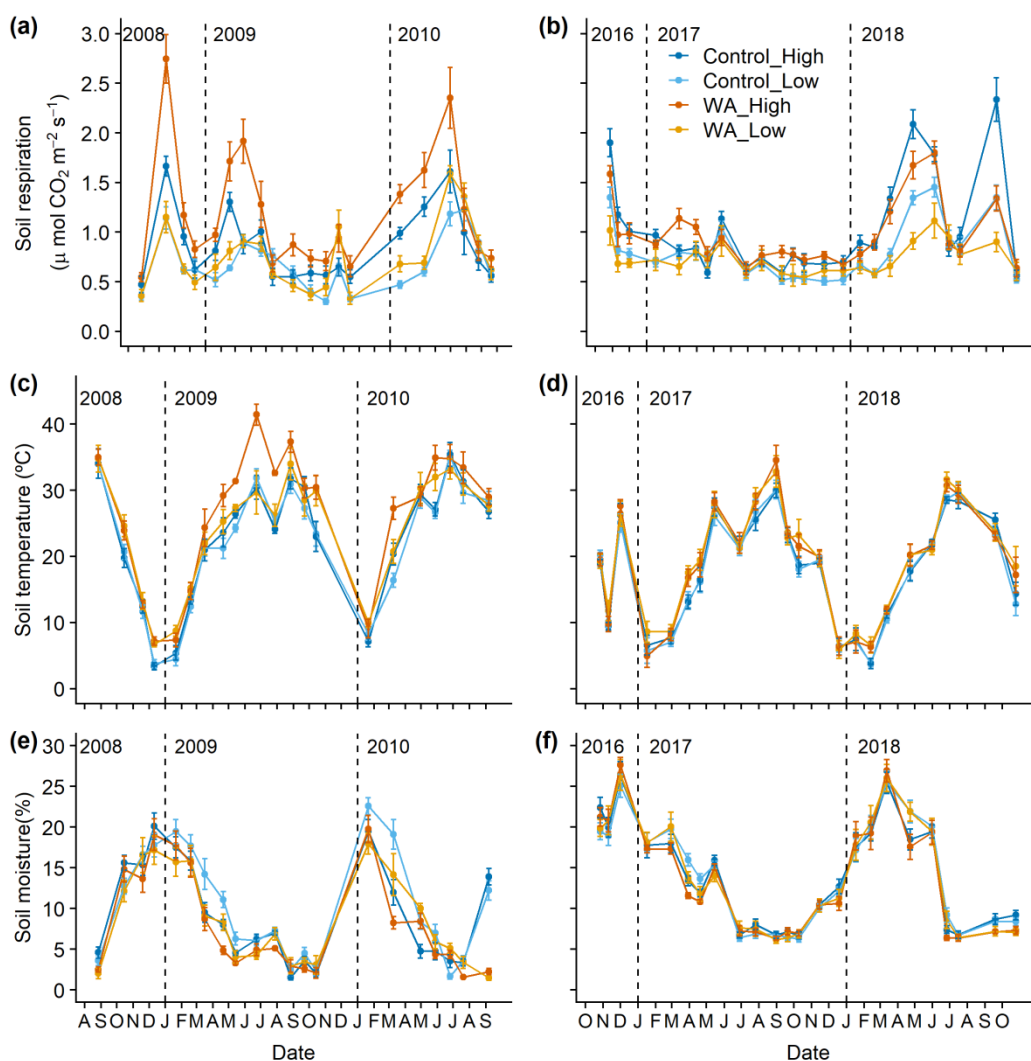
Figure 4. Relationship between the effect size of warming (RR) on soil respiration and on biocrust cover in the short- (a) and longer-term (b). RR data are in ln- scale. The solid line denotes a significant linear model fitted between both variables (R^2 and p values on the graph).

Figure 5. Estimated effects of longer-term warming on potential soil respiration rates at a common soil microbial biomass value and with substrate (glucose) in excess of microbial demand. Effect sizes were estimated using coefficients from the ‘Yeast-SIR’ model (Table S5).

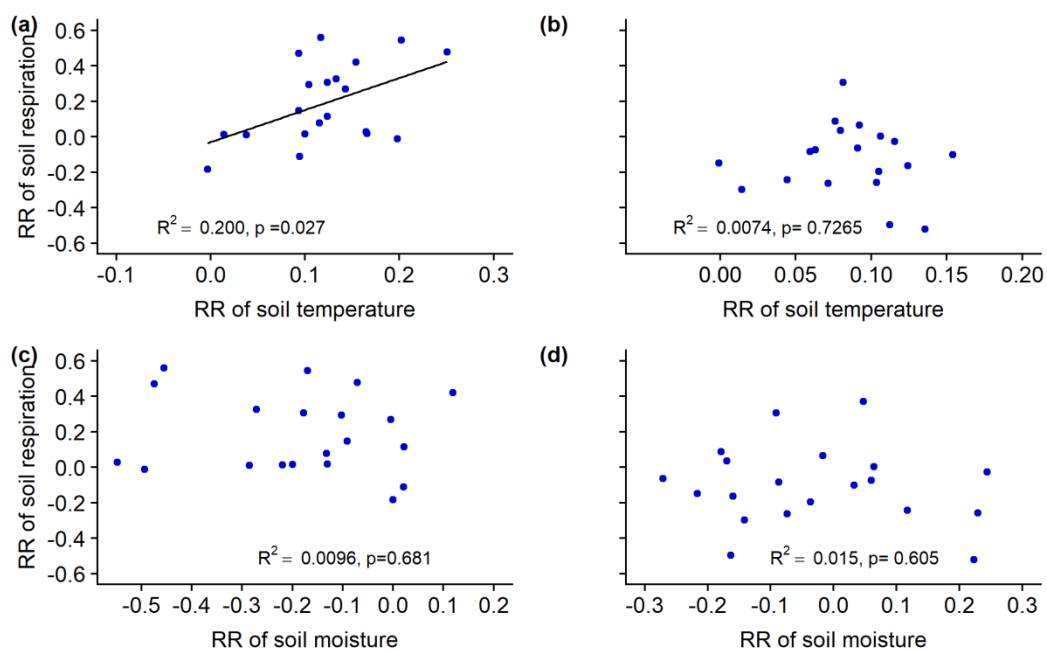
Specifically, the unstandardized coefficients were used in a regression equation, along with the mean of the observed values for microbial biomass, one of the treatments (i.e. control or warming) and one of the assay temperatures (i.e. 10, 20, 30 or 40°C). Given that there were no differences between both initial biocrust cover levels, we only represent the data at the low initial biocrust cover. Error bars show the standard deviation. Asterisks denote significant differences at $p < 0.05$.



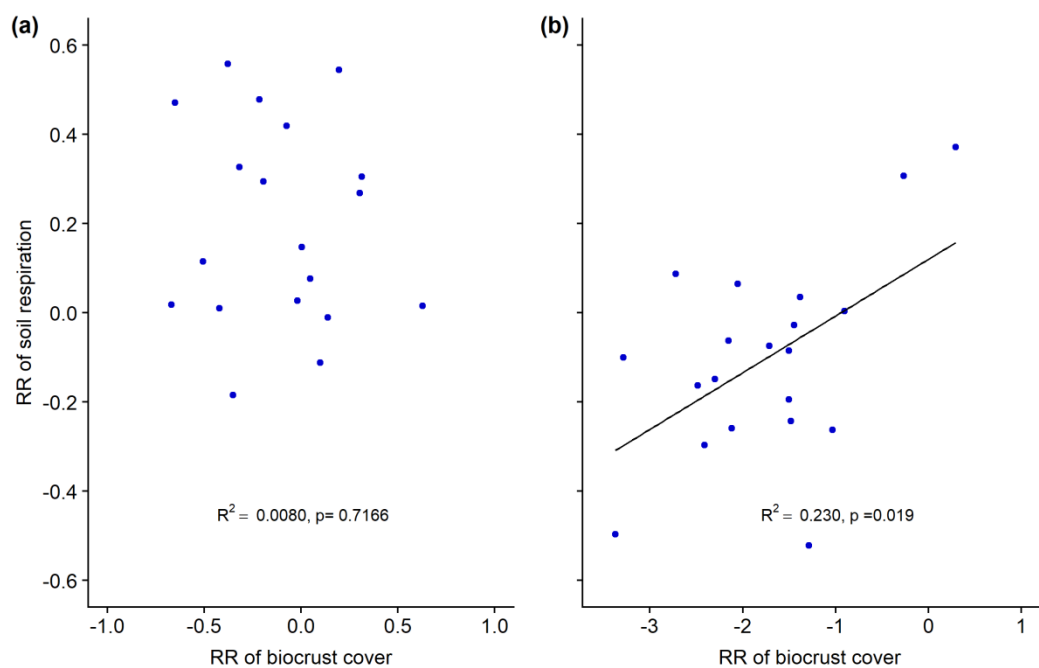
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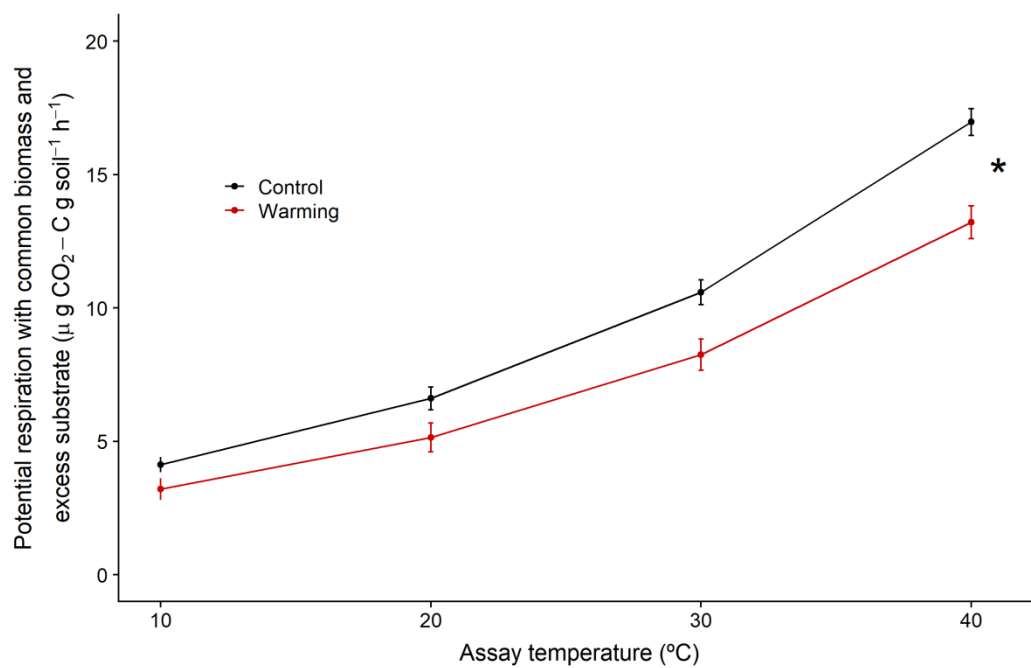
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